PATENT
CASE:JB0600Q

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:	
RAVNIKAR *et al.*	:	Examiner: RAILEY, J.
For Patent For:	:	Group Art Unit: 1636
EXPRESSION OF SOLUBLE HETEROLOGOUS PROTEINS IN BACTERIA UTILIZING A THIOREDOXIN/PROTEIN EXPRESSION VECTOR	:	
Serial No.:08/846,606	:	
Filed: April 30, 1997	:	

Schering-Plough Corporation
Kenilworth, New Jersey 07033

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.131

Sir:

We, Paula D. Ravnikar and Robert Greenberg, declare as follows:

1. That we are the co-inventors of the subject matter disclosed and claimed in the above-identified application;

2. That we are employed by the Schering-Plough Research Institute (SPRI) which is a division of Schering Corporation, the assignee of the above-identified application;

2. That we caused experiments to be carried out in the United States of America which resulted, prior to October 1, 1995, in the construction of a vector containing both a nucleic acid sequence encoding a thioredoxin protein and a nucleic acid encoding a heterologous protein, which vector was capable of causing the expression of the thioredoxin

protein and the heterologous protein as separate, non-fused proteins wherein the heterologous protein was expressed in soluble form;

3. That Exhibits A-C attached to this Declaration are true copies of pages from a permanently bound Notebook numbered 31163, assigned to Paula Ravnikar and maintained at SPRI ("the Ravnikar Notebook");

4. That Exhibit A consists of true copies of pages 20-27 of the Ravnikar Note Book and describes experiments resulting in the cloning of the *E. coli* thioredoxin gene;

5. That Exhibit B consists of true copies of pages 84-89 of the Ravnikar Note Book and describes experiments resulting in the translational coupling, in a single plasmid, of the *E.* coli thioredoxin gene and the human interleukin-13 gene;

6. That Exhibit C, consists of true copies of pages 92-94 and 96-98 of the Ravnikar Note Book and describes experiments demonstrating the expression of human interleukin-13 in bacteria using the coupled translational plasmid the construction of which plasmid is referred to in Exhibit B; and

7. That although the dates on the note book pages referred to in Paragraphs 3-6 have been masked, we hereby confirm that the studies described in those notebook pages were carried out in the United States of America prior to October 1, 1995.

We hereby further declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true, and further, that we make these statements with the

knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Aug 13, 98

Paula D. Ravnikar, Ph.D

Date: Aug 13, 98

Robert Greenberg, Ph.D.

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE U.S. POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON D.C. 20231 ON

August 14, 1998

DATE OF DEPOSIT

IMMAC J. THAMPOE

REGISTERED REPRESENTATIVE

8/14/98

SIGNATURE AND DATE OF SIGNATURE

Exhibit A

298-5388

Thioredoxin : PCR from the coli genome.

Prep coli genomic DNA from a single colony of MM 294 using Biorad instagene = according to Biorad's instructions.

100ul PCR reaction. Run 5ul on gel.

Forward primer for trxA gene with NdeI cloning site

362 trxa.5oli Length: 30 11:13 Type: N Check: 3551 ..

1 CCTGTGGAGT TACATATGAG CGATAAAATT

363 reverse primer for trxA gene with BsaBI and BamHI sites

trxa.3oli Length: 47 11:20 Type: N Check: 9729 ..

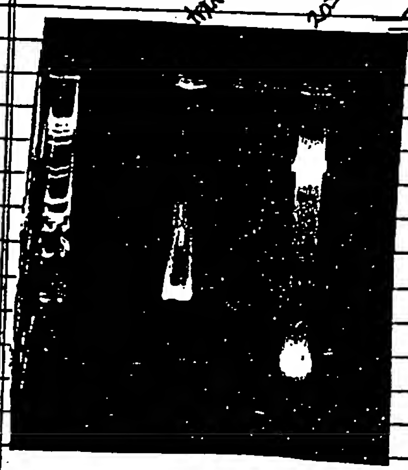
1 GCACCCAACA TGCAAGGATC CTTACCCCAG ATTAGCATGG AGGAACT

Gene clean the PCR Reaction = NdeI + BamHI digest

Isolate from 1.5% gel = Gene Clean - Elute into 50ul.

trxA 202020

Nde.Bam Prep'd Vector



Ligate

Insert 15 ul.

Vector 10 ul

Buffer 5 ul

H_2O 15 ul

Ligase 4 ul

16° overnight

Vector

pMBD 202010

pMBD 202020

Transform 294 / plate on TENK Cm.

Only 202020 yielded colonies

pick 18 to screen.

No correct clones.

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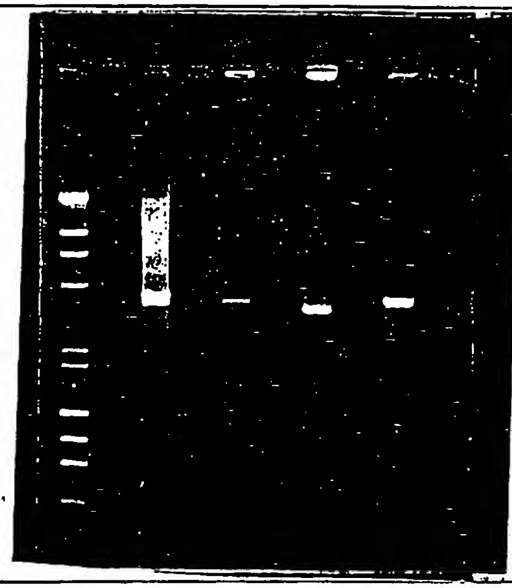
Prep Gel.

① ACYC177 Vela promoter aat/Bam
② 202010 Nde/Bam
③ 202020 Aat/Xba
④ 202080 Nde/Bam
} p-trac.

Gene Clean & elute into 50ul.

after Gene Clean.
① 202020 aat/Xba
② 202020 Nde/Bam
] 3ul.

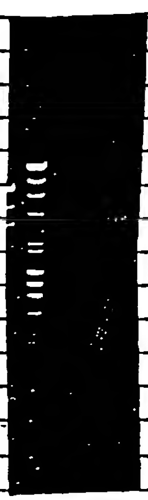
The ACYC177 needs to be Kinased.



ACYC177 pro② / Kinased
100 ul volume final.
4ul Kinase
4ul ATP (100mM stock).
37°C 30min.
Heat inact 65°C/20 min
EtOH ppt.
Resusp 50ul.
run 3ul on Gel.

Ligation

30ul - ACYC177pro
4ul - tripterm
6ul - buffer
20ul - water
-10ul before ligation
+5ul ligase



① ACYC pro②
aat Bam Kinased

② Hirudin
PCR Product
Nde/Bam cut
5ul of 200ul digest

Tripterminator
U374 + U375 (annealed.
180 pm/ul.

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Thio Redoxin PCR Cloning

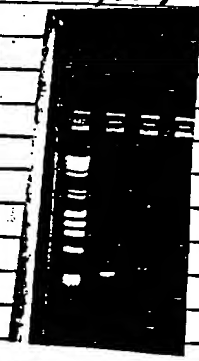
PCR Reaction : Same as from p.20.

NdeI/BamHI digest & Prep frag on 2% agarose.
It separated into 2 bands So the bands were excised & ligated separately.

Prep Gel.

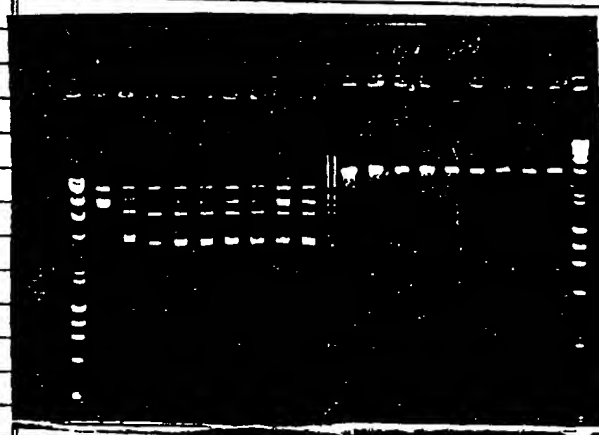
Test after gene clean

After ligation into 20020



~~First~~ Transform 294 & select on Cam plates
The Upper frag gave larger "healthier" looking colonies & were analyzed first.
The lower frag gave smaller & slower growing colonies.

Upper #1-9 There is an NdeI BamHI insert that has no ClaI site (gel #2)
no XmnI site (gel not shown)
XmnI/XbaI gave only a linear vector.



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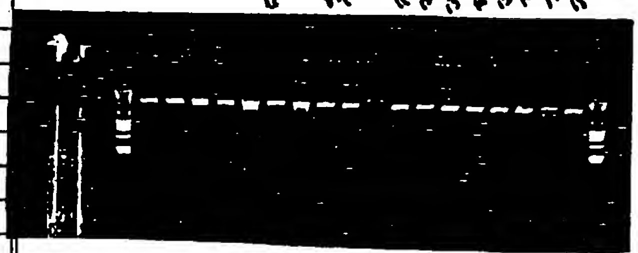
DATE

TxA lower fragment Analysis.

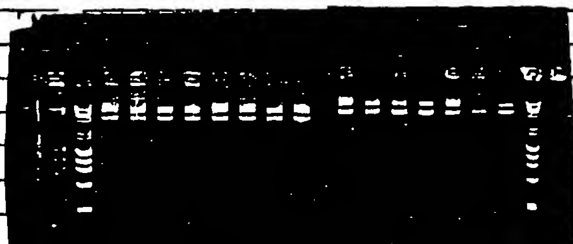
Nde/Bam.

Gel #1



All except #5, 7, 10 have Nde Bam inserts.

Gel #2



ClaI/AatII Digest

No ClaI site in the insert

AatII vector linear.

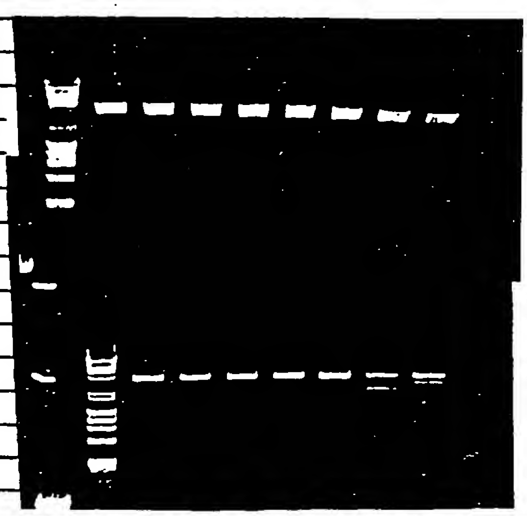
Gel #3

BsaBI Linearized #1, 2, 4, 6, 8, 9, 11, 12, 13, 14, 15, 16

#3, 17, 18 may have an extra site.

BsaBI is part of the oligo used for PCR

Gel #4



XmnI/XbaI Digest

All inserts have an XmnI site.

expect ~325 bp fragment = looks ok.

So far Everything Checks out except the absence of a ClaI site which could be a host strain variation in DNA Sequence.

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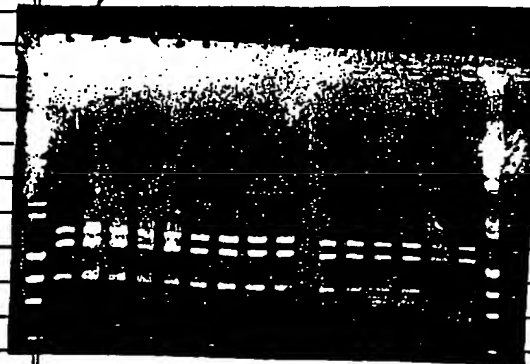
TrxA lower fragment Analysis
ApaLI.

TrxA PCR lower fragment in pMBD202020
pDR475

Gels



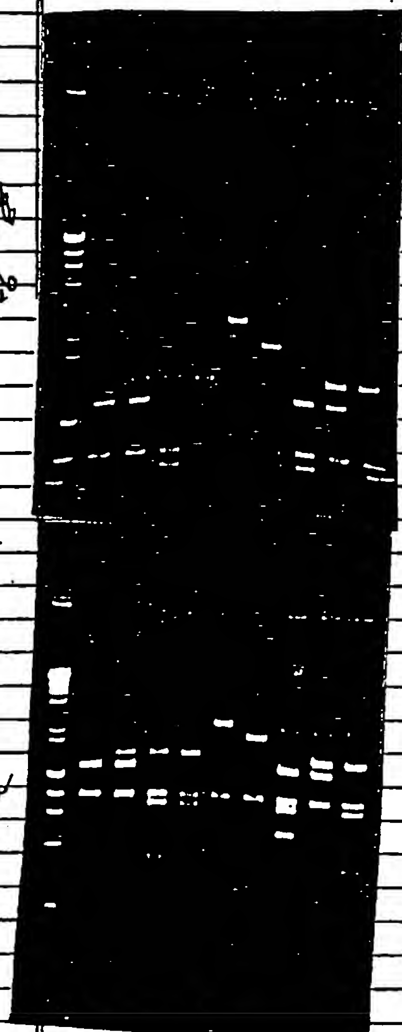
All isolates have 3 ApaLI sites
note: #17 may have 4 sites

Need to verify ApaLI sites in the pMBD202020 vector.

Lrg Scale Prep of #1 & #11

Nov	66
37.5	4360
48	2380
51	2030
64	1350
71	1080

r = -0.4960



Clone #8 66

Clone #6 6a

pMBD202020 vector.

(4) Aat II / Apa
55 - 1821
68 - 1156
71 - 1054

(5) ApaLI
44 - 2935
68 1156

(6) AvaI / ApaLI
48 - 2436
68 - 1156
(+) ~500 bp.

It would appear that the ApaLI site within the terminator at 401 is not present and is the result of a sequencing error or digest variation at that position.

Lanes
(1) Aat / ApaLI
(2) / Apa } Clone #1
(3) Ava / Apa
(4) Aat / Apa
(5) / Apa } 202020 vector
(6) Ava / Apa
(7) Aat / Apa
(8) / Apa } Clone #11
(9) Ava / Apa

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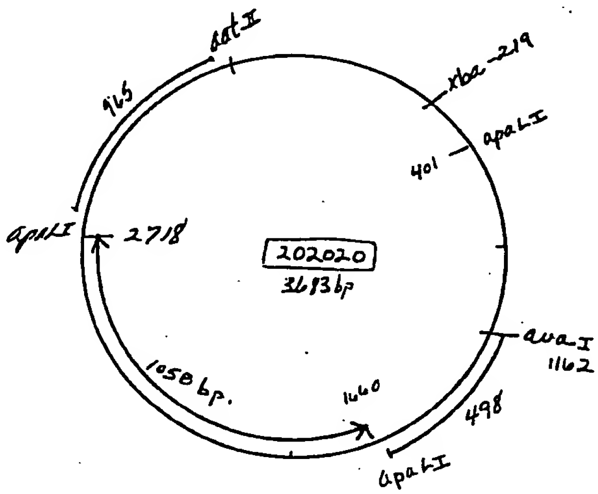
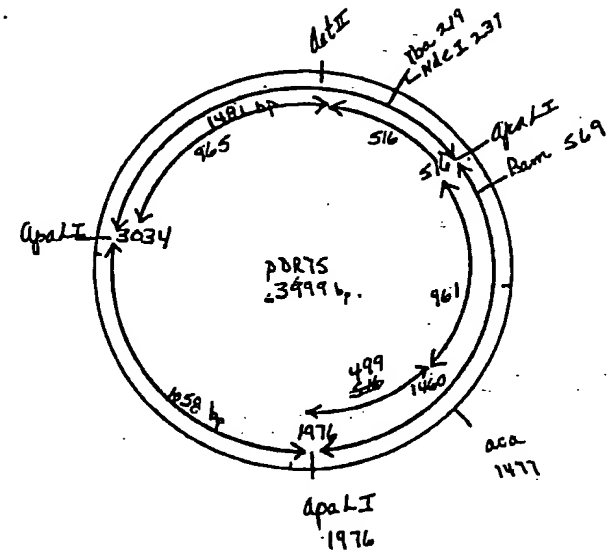
TxA Screen Analysis.





~~(7) Aat Apa~~

λ/Hd3 (6a)

20.5	- 4360
26	- 2380
28	- 2030
35	- 1350
38	- 1080
42	- 870
48	- 600
57.5	- 310

r = -0.9975

aat/Apa 1480 frag ⟶ 965 + 516
(a) largest Apa frag should be cut.
(b) 1796 ⟶ 998 + 762 ok!
insert may be a little larger than thought

ava/Apa 1460 ⟶ 961 + 499
(a) 2nd largest ~~should be cut~~.
(b) 1272 ⟶ 998 + 572.

Clone 11

(7) Aat/Apa

			Expected
32 -	1272		uncut = ok!
37 -	1124		uncut = ok!
39 -	998	4156 total	} from 1796 = ok!
44 -	762		

(8) Apa

30 -	1796		— 1481
32 -	1272	4192	— 1460
37 -	1124		1058

(9) Ava/Apa

30 -	1796		— uncut ok!
37 -	1124		— uncut ok!
39 -	998	4490	} from 1272 = ok!
50 -	572		

(could be 516 bp)

Clone 11 Appears to check out in digest better though insert could be larger than thought.

Clone I ~~digest~~ digest could be screwed up because Apa I does not look like it did in gel #5.

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TrxA pocket contd.

Gel 6a

6b.

Gel 6a + 6b
Different photo exposure from p 24.

Gel #5 p. 24.

λ DNA		AfaI Digest	
28	2326		
31	2030	34	1640
38	1350	36	1465
42	1080	42.5	1057
46.5	870		

r = -0.9977

#11 Sent to Seek for DNA Sequence Confirmation

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one vial of #3,5,7,8 are frozen away in box.

pdr 76.

trxa-IL13 oligo
bsaBI-pshaI/bamHI

trxpsh.oli Length: 47 13:27 Type: N Check: 1217 ..

(384) 1 TAATGTGGCG GGTTCTGGTT CTGGTGATGA CGATGACAAG GGTCCGG

pshAI

Gly Ser Gly Ser Gly D D D D K. AvaII

linker enterokinase

REVERSE-COMPLEMENT of: Trxpsh.Oli check: 8911 from: 1 to: 52

trxa-IL13 oligo
bsaBI-pshaI/bamHI

trxpsh.rev Length: 51 13:28 Type: N Check: 2215 ..

(385) 1 GATCCCGGAC CCTTGTCATC GTCATCACCA GAACCAGAAC CCGCCACATT

51 A

AciI C'CGC

Cuts at: 0 6 47
Size: 6 41

AvaII G'GWC_C

Cuts at: 0 41 47
Size: 41 6

FauI CCCGCnnnn'nn_

Cuts at: 0 1 47
Size: 1 46

HphI GGTGAnnnnnnnn_n'

Cuts at: 0 35 47
Size: 35 12

MspI C'CG_G

Cuts at: 0 44 47
Size: 44 3

NlaIV GGN'NCC

Cuts at: 0 42 47
Size: 42 5

PshAI GACnn'nnGTC

Cuts at: 0 39 47
Size: 39 8

Sau96I G'GNC_C

Cuts at: 0 41 47
Size: 41 6

BsaBI-pshAI/BamHI oligo.

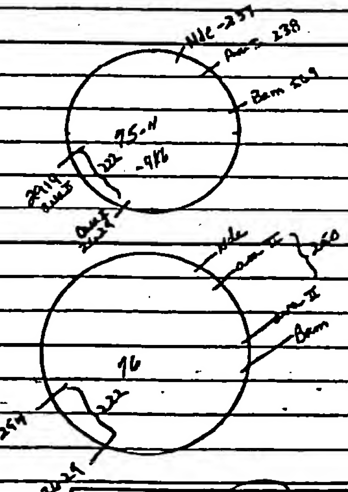
encodes end of trxA + linker + enterokinase cleavage site — and will be cloning sites for future coupled sequences
Anneal oligos
Ligate to pDR75-11 BsaBI/BamHI cut.

Transform 294 + select on Amp plates.
Magic Mini Screen

75-11 Prep Gel.

Digest with Ava II

Unfortunately a nucleotide error in the oligo destroys the BsaBI site and changes codon in the end of trxA (leu → val).
Make a new & correct oligo.



Clones 3+7 look correct. Digest on #7 looks better.
Have 220/250 Doublet & ~900 bp frag. is ~650 w/ 250 bp shorter.
Note that all the Ava II sites in 20020 appear to be incompletely mapped as yet.

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Exhibit B

p. 34 u471 oligo sequence used to make the trx-IL13 fusion

3'-5' oligo for trx-il13 coupled translation

u468.oli Length: 58 11:34 Type: N Check: 7052 ..

1

>SEQED (include) of: u468.oli check: 671 from: 1 to: 68>

CACGCACAGC GGTAGACGGC GGAACGGGAC CCATGGAATA CCTCCTTAAG

51 CCAGATTT

<SEQED (include) of: u468.oli check: 671 from: 1 to: 68<

coupled translation oligo for trx-il13

u467.oli Length: 62 11:30 Type: N Check: 1430 ..

1

>SEQED (include) of: trxl13cup.seq check: 4225 from: 1 to: 68>

AAATCTGGCT TAAGGAGGTA TTCCATGGGT CCCGTTCCGC CGTCTACCGC (NcoI) (RBS)

51 TCTGCGTGAG CT

<SEQED (include) of: trxl3cup.seq check: 4225 from: 1 to: 68<

oligos. 467/468 pDK100

This reduces hIL13

coupled translation

oligo: BsaBI → SstI

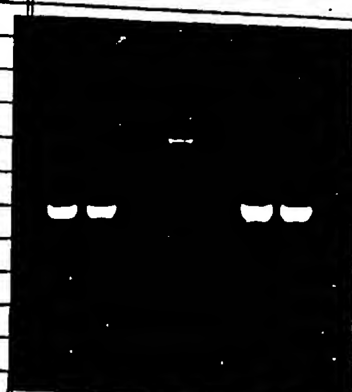
Insert into pDE99/Hy

derivative of pDE88.

467/468 oligo puts an NcoI site at the ATG of IL13 for future cloning use

pDE88] pDE99] BsaBI

BsaBI appears to be unique in both plasmids.



pDE88 Prep Gel.

BsaBI/SstI

PshAI/SstI

annealed Oligos on a 4% NuSieve Gel

① 467/468 58mer

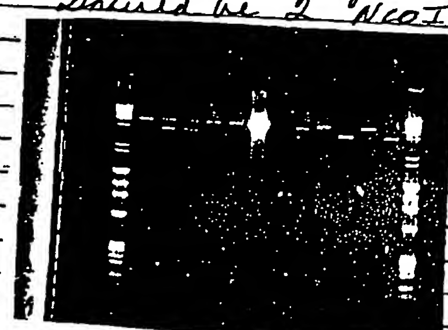
② 469/470 47mer

③ 471/472 50 mer.

④ 100 bp ladder

Screen NcoI digest

Should be 2 NcoI Sites



No positive clones

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Construction of pDR100 + pDR101 by annealing oligos + ligation
has not proceeded well. alternative: PCR modify IL13.

forward oligos: U478 + U479
Reverse oligos: U361 + U406 : both are downstream of BamHI site
of an IL13 clone.
Template: pDR88
Clone PCR products as BsaBI/BamHI fragments into pDR88.

trxA-hIL13 translational coupling
PCR primer BsaBI cloning site
RBS overlaps the TAA stop codon
NcoI site introduced at the ATG
U478.oli Length: 71 09:28 Type: N Check: 8193 ..

Similar to U467

1 TTGAAAGAGT TCCTCGATGC TAATCTGGCG TAAGGAGGTA TTCCATGGGT

51 CCGGTTCCGC CGTCTACCGC T

trxA-hIL13 translational coupling
PCR primer BsaBI cloning site
PshAI site removed//ApaI site added atG GGC CCg

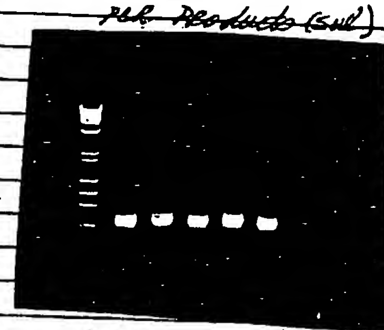
Similar to U469.

u479.oli Length: 72 09:34 Type: N Check: 9076 ..

1 TTGAAAGAGT TCCTCGATGC TAATCTGGCG TTGGAGGATG ATTAAATGGG

51 CCCGGTTCCG CCGTCTACCG CT

~~PCR Products~~ (5ul)



1 - λBX
2 - 361+406 }
3 - 361 } +478
4 - 406 }
5 - 361 } +479
6 - 406 }

see Gel.

1 361/478 }
2 361/479 } BsaBI
3+4 pDR88 } BamHI.

Ligation
Vector - 10
insert - 10
Buffer - 2.5
Ligase - 2.5
25ul.

Transform w/ 294
Select on Amp plates.

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Thioredoxin - h IL13 Coupled translation   oligo 469/470   1"

Trx - IL13 fused plasmid has a sequence:

asp asp asp lys gly pro   pro pro

GAC · GAT · GAC · AAG · GGT · CCG · GTT · CCG · CCG · etc

GAC nnnnnGTC

pshAI

Make a pshAI - SstI oligo to couple translation

asp asp asp — S.D.

GAC · GAT · GAC · AA | C · GAG · GAT · GAT · TAA · ATG · GGT · CCG · etc

asn glu asp asp * met gly pro

This arrangement differs from 467/468 in the following

1) Shine Dalgarno is within a translated region
2) TAA · ATG stop & start are immediately adjacent.
3) Coupling utilizes a trx protein very similar to the one used in the fu

REVERSE-COMPLEMENT of: U470.oli check: 4070 from: 1 to: 51

REVERSE-COMPLEMENT of: U369.oli check: 6198 from: 1 to: 51
coupled translation oligo
pshai-ssti linker

U469.oli Length: 51 10:24 Type: N Check: 6198 ..

1 CGAGGATGAT TAAATGGGTC CGGTTCCGCC GTCTACCGCT CTGCGTGACC

51 T

REVERSE-COMPLEMENT of: U369.oli check: 6198 from: 1 to: 51

coupled translation oligo
pshai-ssti linker

u470.oli Length: 47 10:26 Type: N Check: 142 ..

1 CACGCAGAGC GGTAGACGGC GGAACCGGAC CCATTTAATC ATCCTCG

Tried to dig a small Xma I / SstI fragment.
There did not appear to be any positive clones.

Repeated ligation: screen with Xba · Bam & look for 930 bp fragment.

clone # 26 - 29 - 30 - 34 - 36 looked positive
screen done by John L. Book # 32780 p 66.

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[illegible] for plasmid [illegible]

#26-29-30-34-36. [illegible] colony inoculated 25 ml IFN KR
[illegible] @ 30°C for 7hrs. 5 ml removed to tubes [illegible]
to make stock frozen cultures.
Remainder added IPTG (10 mM) [illegible] ON @ 42°C.

	Abs	10 ml
#26	1000	2.0
#29	950	1.9
#30	1250	2.4
#34	1150	2.25
#36.	1550	3.0

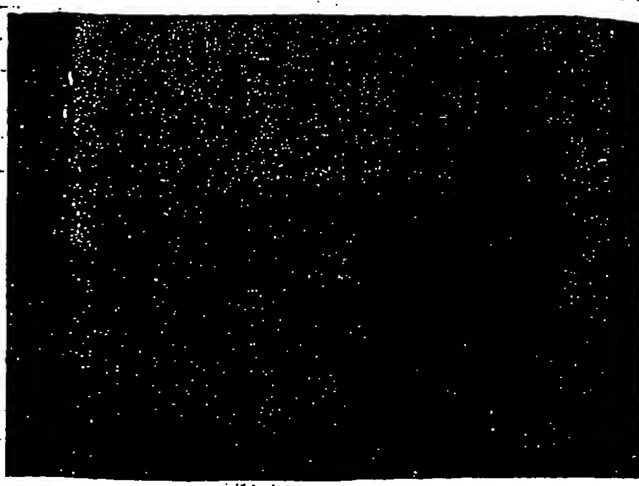
[illegible] that cultures were induced around 30-50 Klett.
All cultures are [illegible] cells & completely filled with strings of [illegible]

Adds Cell SLP to 30 OD/ml

Western in [illegible] Notebook #32780 p 70

SLP to 30 OD & 10 ul per lane.

1. [illegible]
2. 16.13 = 50 ng
3. HMS174 pLE4I 37° 5hr
4. 42° 5hr
5. 37° ON
6. 42° ON
7. HMS174 pLETS 37 5hr
8. 42 5hr
9. 37 ON
10. 42 ON
11. 101-26
12. 101-29
13. 101-30
14. 101-34
15. 101-36.



clones still look like [illegible] fusions

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Thioredoxin -IL13 Coupled Translation Oligos 471/472

pDR107

REVERSE-COMPLEMENT of: U472.oli check: 906 from: 1 to: 50

REVERSE-COMPLEMENT of: U471.oli check: 7686 from: 1 to: 54
trx-il13 coupled translation linker
bsabi-ssti
u471.oli Length: 54 ... 14:45 Type: N Check: 7686 ..

1 GAAGGAGGCT GATTAAATGG GTCCCGTTCC GCCGTCTACC GCTCTGCGTG
(R.B.S.)

51 AGCT

REVERSE-COMPLEMENT of: U471.oli check: 7686 from: 1 to: 54

u472.oli Length: 50 ... 14:44 Type: N Check: 906 ..

1 CACGCAGAGC GGTAGACGGC GGAACGGGAC CCATTTAATC AGCCTCCTTC

BsaBI

GAT nnnnATC

GAT·GCG·AAT·CTG·GCG·TAA
asp ala asn leu ala end

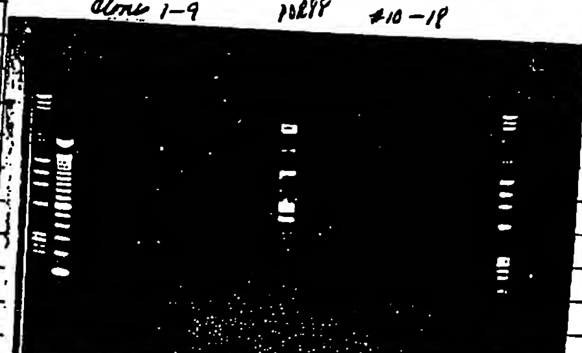
GAT·GCG·AAG·GAG·GCT·GAT·TAA·ATG ....> IL13/SSTI
GAAGGAGG ←8bp→
asp ala lys glu ala asp end met

The coupled system above
1) introduces ribosome binding site GAAGGAGG into the C terminus of the native thioredoxin protein. Amino acid changes in trx were made as necessary to introduce a strong R.B.S.

BsaBI site is 1st.
BsaBI / BsaHI digest

BsaHI: 2 —759 (BsaBI)— 2659 —— 3342 —— (6871) — 2
2657 └ 683 ┘ └ 3530 ┘

clones 1-9 pDR98 #10-18



EcoRI NruII NcoI Bam

EcoRI 2920, 2712

Nru II 2350, 2280
1290, 734
108 + 93

Different to Sev & Need Full DNA.

Nco/Bam = 97

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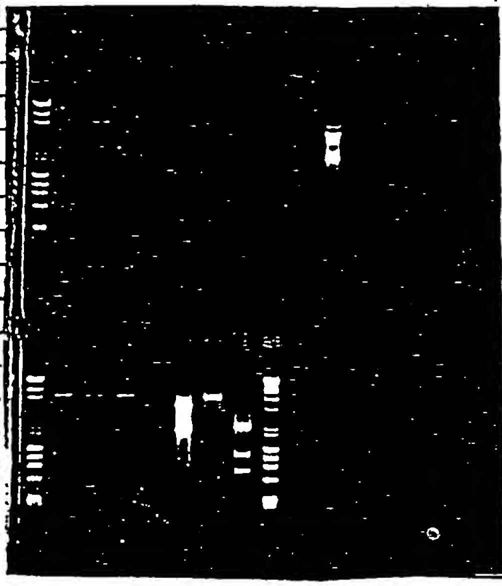


BsaB1/BstE II digest
Should be Green plasmid
6870 bp.
#2-4-5-10-13-15-16-17-18
look positive.

check SstI site since it is the
other cloning site.

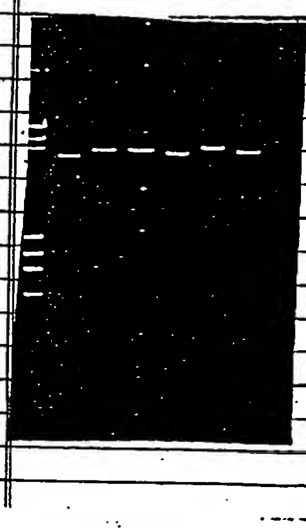
SstI/EcoRV digest.

SstI/RV digest
#2-5-13-15-16 maybe 17-18 look positive.

Check fermentations for appearance of
monomer etc.
See p. 92-96

Fermentations show 4-5-10-~~13~~-15-16-17-~~18~~
to be positive.

Prepped #13 & #15 for more DNA.



SstI/EcoN I
pshA1/BamH I } #13
XbaI/BamH I
SstI/EcoN I
pshA1/BamH I } #15
Xba/BamH I

#13 has no Xba-Bam
fragment.

pDF18 EcoN I @ 1323
SstI @ 834
total 6875 bp
should be 490 bp fragment.

Note there seems to be an
extra EcoN I site approx 700 bp
in #15
The psh A1 site is gone &
Xba-Bam frag is the
appropriate size.

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Exhibit C

Thioredoxin - IL13 fermentation

Time of induction

pDR88 ; pDR99 ; pLET.
Cam Ap Cam/C.

cell chip → flask slow aeration overnight IFN γ

→ pDR88 | Inoculate @ 30, 90, 200 x.
→ pDR99 | Inoculate @ 30 x

IFN 16 R
100ml / 500 ml flask

Grow @ 30°C / 250 rpm a few hours.
Induce w/ 100 μM IPTG and grow @ 15°C / 250 rpm

→ pLET1 | Inoc @ 30x and grow at 15°C / 250 rpm

	Induced @ OD	18 hrs	43 hrs	67 hrs *
pDR88	30 - 150	770	2300	3000
	90 - 275	1150	2650	3500
	200 - 600	1650	3150	3100
pDR99	30 - 130	700	1900	3150
pLET1	30 x	170	1375	3400

at 43 hrs: Take a 25ml sample + replace cultures at 15°C/250 rpm.
Resusp to 30 OD/ml in TEN buffer

insoluble white-green tube

1. Sonicate SN → green + yellow labels.
2. whole cell GRP → violet labels.
3. Osmotic shock → purple + magenta
4. Heat denaturation → red + pink.

Osmotic Shock
1ml of 30 OD/ml.
↓ spin
Resusp 500ul TE (40:5)
Add 500ul 40% Sucrose.
↓ ice 10min
Spin 30sec
↓
Resusp in 1ml TE (40:5)
↓ ice 10min
Spin 5min
Shockate → Sup @ 30 OD → purple labels
Pellet ↓ GRP to 30 OD → magenta labels

Heat denaturation

Samples pDR88 / 30x inoculum
pDR99
pLET I.

500ul of unseparated lysate
heat 0 - 2 - 5 - 10 min
spin 10 min
Sup → SN. → red labels
Pellet → GRP to 500ul final. → pink labels

* Samples from 67 hrs are labeled as day 2 : Only have SN/insol sonicated fraction

After 67 Hours : Microscopic examination:
pDR88 = saw alot of short fat cells ~50% have inclusion bodies
pDR99 = short fat cells, not many inclusion bodies
pLET I = looked like a normal E. coli Host & not producing recombinant proteins. Cells are short thin rods.

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GEL I: Soluble

1	Size std	
2	294 Host	
3	rbuIL-13 std (CHO) 50 ng	
4	pDR88-150	43 Hrs
5	pDR88-275	
6	pDR88-600	
7	pDR99-130	
8	pLET1-30	
9	pDR88-150	67 Hrs
10	pDR88-275	
11	pDR88-600	
12	pDR99-130	
13	pLET1-30	

Gel II:

1	Size Std	
2	pDR88-150	Shocknate (Sol.)
3	pDR88-275	
4	pDR88-600	
5	pDR99-130	
6	pLET1-30	
7	pDR88-150	Shock pellet
8	pDR88-275	
9	pDR88-600	
10	pDR99-130	
11	pLET1-30	
12	pDR88-150	Whole Cell GRP
13	pDR88-275	
14	pDR88-600	
15	pDR99-130	

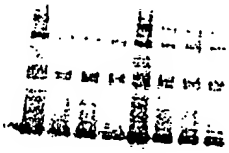
Gel III: Insoluble Fractions

1	Size Std	
2	pDR88-150	43 Hrs
3	pDR88-275	
4	pDR88-600	
5	pDR99-130	
6	pLET1-30	
7	pDR88-150	67 Hrs
8	pDR88-275	
9	pDR88-600	
10	pDR99-130	
11	pLET1-30	
12	pDR88-150	10' Heat Shock Pellets
13	pDR99-130	
14	pLET1	
15	rbuIL-13 Std (CHO) 50 ng	

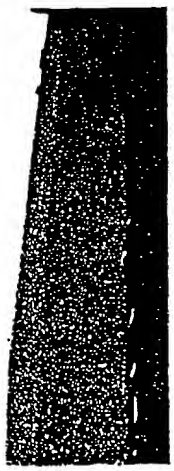
Gel IV: Soluble Heat Shock Fractions

1	Size Std	
2	pDR88-150	0'
3	pDR88-150	2'
4	pDR88-150	5'
5	pDR88-150	10'
6	pDR99-130	0'
7	pDR99-130	2'
8	pDR99-130	5'
9	pDR99-130	10'
10	pLET1-30	0'
11	pLET1-30	2'
12	pLET1-30	5'
13	pLET1-30	10'
14	IL13 std.	

Extra photos of gels 1-4 in file box



4



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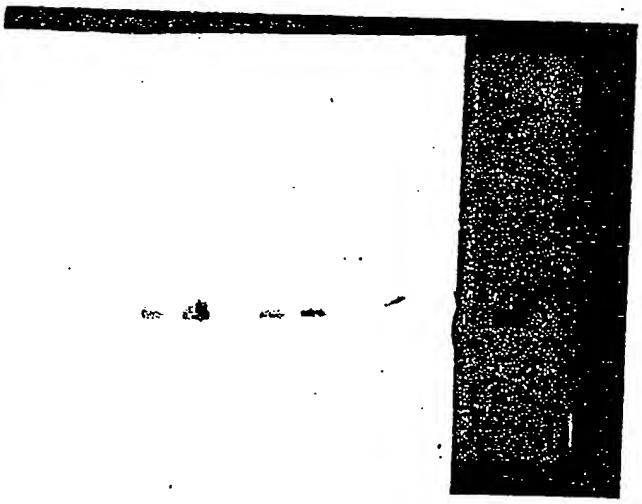
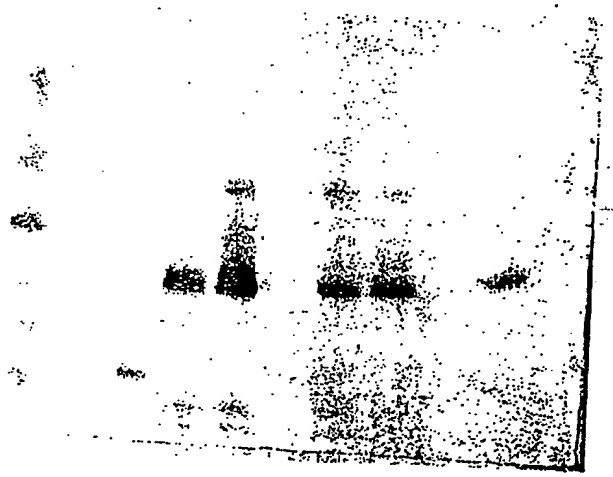


5 ul 30 OD before
2.5 ul 15 after dye
Use Kilodaltype
sheet
1kb = 50 ng
pDR86 - 5ul 50D
pDR99 - 5ul 50D
plut1 - 10ul 30OD
pDR86 - 5ul 50D
pDR99 - 5ul 50D
plut1 - 10ul 30OD
pDR99 - 5ul 50D
5ul after 10min/80°C



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Thio Reductase - IL13 Coupled Translation
471/472 oligos pBL 102

Fermentation

Single colony isolated clones from p. 88 Seed → 25 ml IFN IL-2 broth
Grew @ 30°C 5 hrs — Induce w/ 100 uM IPTG & upshift to 41°C
Grew ON GLP samples to 30 OD.

#2 - 1800
4 - 950
5 - 750
10 - 775
13 - 1900
15 - 1050
16 - 750
17 - 850
18 - 1950

2 shorter cells w/ polar dark spots
4 extremely long cells - snakes - 2-8 light refractile IBs
5 ~50% as 4 & 50% long cells w/ 2 LR IBs per cell
10 Same as 4
13 - Short cells polar dark spots
15 - long cells typically 1 LR IB per cell
16 - Same as 4
17 - Same as 4
18 - Very short stubby cells ~50% have 1 R IB.

1 Sig Std + IL13 E.coli Std
2 #2 ~~Blank~~
(A) 3 #4 / 4 #5 / 5 #10 — positive
6 #13
(B) 7 #15 / 8 #16 / 9 #17 — positive
10 #18

4-5-10-15-16-17 are positive

(A) Do have Cotranslation
(B) Do have Accumulation @ 42°C

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single colony Isolate from ...
↓
Single Colony Isolate again
↓
Single colony → 10ml IFN8 + Km. Start Growing at 30°C.
↓ 5ml.
Inoculate 50ml in a 310ml baffled flask.
IFN 16 Z Cam.
Grow at 30°C ~3 hrs.

Add 100 mM IPTG & shift to 15°C.

Induce at 2:30					
...	Klett	21 hrs	48 hrs	68 Hrs	
4	45	450	2100	3350	short cells maybe polar dark spots faint
5	55	525	2300	3500	starting to form
10	30	350	1550	3000	Cells ~50% elongated (not snakes) maybe polar dark spots
15	65	350	1400	3000	shortish cells possible dark spots.
16	70	625	2700	3600	Cells are quite short look a little Grainy
17	25	825	3150	3800	Cells are short & otherwise unremarkable.

At 48 hrs. Took a 13ml Sample & froze pellet for analysis.
Plated #15, #17 for plasmid Stability

at 48 hrs no sign of inclusion bodies. Cells were short but longer & thicker than a ... type E. coli.

Stability 48 hrs.

pDR102-15

...	+Cam	IFN8		+Cam	IFN8
10^{-8}	37	25	10^{-7}	171	160
	46	57		252	151
	N.D	17		423	311
	83+?	99			

41.5 / 33 = 126% ; 3.3×10^9

136% ; 1.55×10^{9}

126% / 136%

1400 K = 2.4×10^9

pDR102-17

	+Cam	IFN8
10^{-8}	92	179
	86	219
	152	145
	330	543

61%

10^{-7} & 10^{-6} plates were too crowded to count.

61%
3150 K = 5×10^{10}

pDR102-15 Colonies were small, glossy, round. found 2 larger flatter shaped clones. These were ... as 102-15-101 & 102-15-102

pDR102-17 All Colonies were larger, flatter in morphology.

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pDR102

(Extra Photo Arc in Drx)

Sol of 5 OD.

S-1 pDR102

1	Std		
2	Std 202020		
3	IL13		
4	pDR102-4		48 hr. sol.
5		5	
6		10	
7		15	
8		16	
9		17	
10	pDR102-4		28 hr. sol.
11		5	
12		10	
13		15	
14		16	
15		17	

Gel 2: insoluble

Sol of 30 OD

Sol

1	Sige		
2	202020 std		
3	IL13 100ng		
4	pDR102-4		48 hr
5		5	
6		10	
7		15	
8		16	
9		17	
10	pDR102-4		68 hr
11		5	
12		10	
13		15	
14		16	
15		17	

Looks like monomer IL13 & is soluble.

asp ala asn leu ala
TIX 13: GAT·GCT·AAT·CTG·GCG·TAA
asp·ala·Lys·Glu·ala·asp * met
pDR102 GAT·GCG·AAG·GAG·GCT·GAT TAA·ATG ..... (IL13) →
R.B.S. ← 8 bp → met

TAA
ATG

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